Supplementary Figure Legends:

Supplementary Figure S1. OGT and O-GlcNAc not regulated by loss of cell adhesion in cancer cells. A. Cell lysates from MCF7 cells attached (Adherence +) or placed in cell suspension (anoikis) (Adherence -) for 24 or 48 hours in regular media were collected for immunoblot analysis with the indicated antibodies. **B.** Cell lysates from MDA-MB-231 cells attached (Adherence +) or placed in cell suspension (anoikis) (Adherence -) for 24 or 48 hours in regular media were collected for immunoblot analysis with the indicated antibodies.

Supplementary Figure S2. OGT inhibition attenuates mammosphere formation and $CD44^{H}CD24^{L}$ TIC population. A. Cell lysates from MCF7 cells expressing control or OGT shRNA were collected for immunoblot analysis with the indicated antibodies (left). Representative images of mammospheres (middle) and quantification of mammosphere forming efficiency (MFE) (right) in MCF7 cells stably expressing control and OGT shRNA. **B.** Representative flow cytometry analysis of $CD44^{H}CD24^{L}$ population (left) in MCF7 cells stably expressing control, OGT-1 shRNA and quantified (right)**. C.** Cell lysates from SUM159 cells expressing control or OGT shRNA were collected for immunoblot analysis with the indicated antibodies (left). Representative images of mammospheres (middle) and quantification of mammosphere forming efficiency (MFE) (right) in SUM159 cells stably expressing control and OGT-1 and OGT-2 shRNA. **D.** Representative flow cytometry analysis of $CD44^{H}CD24^{L}$ population (left) in SUM159 cells stably expressing control, OGT-1 shRNA and quantified (right). **E.** Cell lysates from MDA-MB-157 cells expressing control or OGT shRNA were collected for immunoblot analysis with the indicated antibodies (left). Representative flow cytometry analysis of $CD44^{H}CD24^{L}$ population (middle) in MDA-MB-157 cells stably expressing control, OGT-1 shRNA and quantified (right).

F. Cell lysates from SUM159 cells expressing control or OGT shRNA were collected for immunoblot analysis with the indicated antibodies (left). Annexin/PI staining measuring early and late apoptosis in SUM159 mammospheres upon OGT knockdown (right). Student's t-test reported as mean ± SEM. * = p-value < 0.05.

Supplementary Figure S3. OGT inhibition attenuates mammosphere formation and $CD44^{H}CD24^{L}$ TIC population. A. Cell lysates from MCF7 cells treated with DMSO and OGT inhibitor Ac-5s-GlcNAc (100 μM) **(**left) were collected for immunoblot analysis with the indicated antibodies. Representative mammosphere images (center) and quantified MFE post 7 day in mammosphere culture (right). **B.** Same as in A except for using SUM159 cells. **C.** Same as in A except using HCI-10 cells except MFE post 5 day in mammosphere culture. Student's t-test reported as mean ± SEM. * = p-value < 0.05.

Supplementary Figure S4. Elevated OGT and O-GlcNAc ameliorate mammosphere formation and $CD44^{H}CD24^{L}$ TIC population. A. Cell lysates from MCF7 cells stably overexpressing control or OGT were collected for immunoblot analysis with indicated antibodies (left). Representative images of mammospheres formation of MCF7 cells stably overexpressing control or OGT (middle) and quantification of MFE (right). **B.** Representative flow cytometry measuring $CD44^{H}CD24^{L}$ population from MCF7 cells stably overexpressing control or OGT (left) and quantification (right). **C.** Same as in A except using SUM159 cells. **D.** Same as in B except using SUM159 cells. **E.** Cell lysates from MCF7 cells treated with DMSO and OGA inhibitor Thiamet-G (1 μM) were collected for immunoblot analysis with indicated antibodies **(**left),

representative mammospheres formation (center) and quantified MFE following 7day mammosphere culture (right). Student's t-test reported as mean ± SEM. * = p-value < 0.05.

Supplementary Figure S5. OGT/O-GlcNAc modulation affects NANOG-GFP+ and ALDH+ TIC population. A. Cell lysates from SUM159 cells expressing control, OGT-1, or OGT-2 shRNA were collected for immunoblot analysis with the indicated antibodies (top). Quantified flow cytometry graph showing NANOG-GFP+ population in SUM159 cells stably expressing control, OGT-1 or OGT-2 shRNA (bottom). **B.** Same as in A except for using MDA-MB-157 cells. **C.** Cell lysates from MCF7 cells expressing control or OGT-1 shRNA were collected for immunoblot analysis with the indicated antibodies (top). Quantified flow cytometry graph showing NANOG-GFP+ population in MCF7 cells stably expressing control or OGT-1 shRNA (bottom). **D.** Quantified flow cytometry graph showing NANOG-GFP+ population in SUM159 cells stably overexpressing control or OGT. **E**. Cell lysates from MDA-MB-157 cells stably overexpressing control or OGT were collected for immunoblot analysis with indicated antibodies (top). Quantified flow cytometry graph showing NANOG-GFP+ population in MDA-MB-157 cells stably overexpressing control or OGT (bottom). Student's t-test reported as mean ± SEM. * = p-value < 0.05.

Supplementary Figure S6. OGT/O-GlcNAc modulation affects NANOG-GFP+ TIC population. A. Cell lysates from MDA-MB-231 cells treated with DMSO or OGA inhibitor NButGT (100 μM) were collected for immunoblot analysis with indicated antibodies **(**left). Quantified flow cytometry graph showing NANOG-GFP+ population in MDA-MB-231 cells following 48hr treatment with DMSO or NButGT (100 μM) (right). **B.** Same as in A except using

SUM159 cells. **C.** Same as in A except using MDA-MB-157 cells. **D.** Same as in A except using MCF7 cells. **E.** Quantified flow cytometry graph showing ALDH+ population in MDA-MB-231 cells stably expressing shRNA control or OGT shRNA. **F.** Quantified flow cytometry graph showing ALDH+ population in MCF7 cells stably expressing shRNA control or OGT shRNA. **G.** Quantified flow cytometry graph showing ALDH+ population in MDA-MB-157 cells stably expressing shRNA control or OGT shRNA. Student's t-test reported as mean ± SEM. * = p-value < 0.05.

Supplementary Figure S7. OGT regulates EMT and stem cell markers. A. Quantification of fold change in indicated proteins levels in MCF7 cells expressing shRNA control or OGT shRNA**. B.** Quantification of fold change in indicated proteins levels in MCF7 cells overexpressing control or OGT**. C.** Quantification of fold change in indicated proteins levels in MDA-MB-231 cells expressing shRNA control or OGT shRNA**. D.** Quantification of fold change in indicated proteins levels in MDA-MB-231 cells overexpressing control or OGT**. E.** Quantification of fold change in indicated proteins levels in SUM159 cells expressing shRNA control or OGT shRNA**. F.** Quantification of fold change in indicated proteins levels in SUM159 cells overexpressing control or OGT**.**

Supplementary Figure S8. OGT regulates EMT and stem cell markers.

A. Measurement of relative mRNA expression of indicated genes from control or stable OGT shRNA expressing MDA-MB-231 cells using qRT-PCR. All expression is normalized to cyclophilin A internal control and to MDA-MB-231 shRNA control samples. **B.** Measurement of relative mRNA expression of indicated genes from control or stable OGT overexpressing MDA-

MB-231 cells. **C.** Measurement of relative mRNA expression of indicated genes from control or stable OGT shRNA expressing SUM159 cells. **D.** Measurement of relative mRNA expression of indicated genes from control or stable OGT overexpressing SUM159 cells. Student's t-test reported as mean ± SEM. * = p-value < 0.05.

Supplementary Figure S9. KLF8 is a critical regulator of mammosphere formation and required for OGT-mediated mammosphere growth. A. Pathway enrichment plot with pathway names on X-axis and number of differentially expressed genes on Y-axis: upregulated genes (red), downregulated genes (blue). **B.** Cell lysates from MDA-MB-231, HCI-10, MCF7 cells grown in adherent (adh) and mammosphere (mamm) culture were collected for immunoblot analysis with indicated antibodies. **C.** Cell lysates from SUM159 cells stably expressing control or two different KLF8 shRNA were collected for immunoblot analysis with indicated antibodies (top), representative mammosphere images (bottom). **D.** Cell lysates from MDA-MB-231 cells stably expressing control or two different KLF8 shRNA were collected for immunoblot analysis with indicated antibodies (top) and quantified MFE in KLF8 knockdown in MDA-MB-231 cells using two shRNA constructs (bottom). **E.** Cell lysates from SUM159 cells with KLF8 knockdown treated with DMSO or NButGT (100 μM) for 48hrs were collected for immunoblot analysis with indicated antibodies.

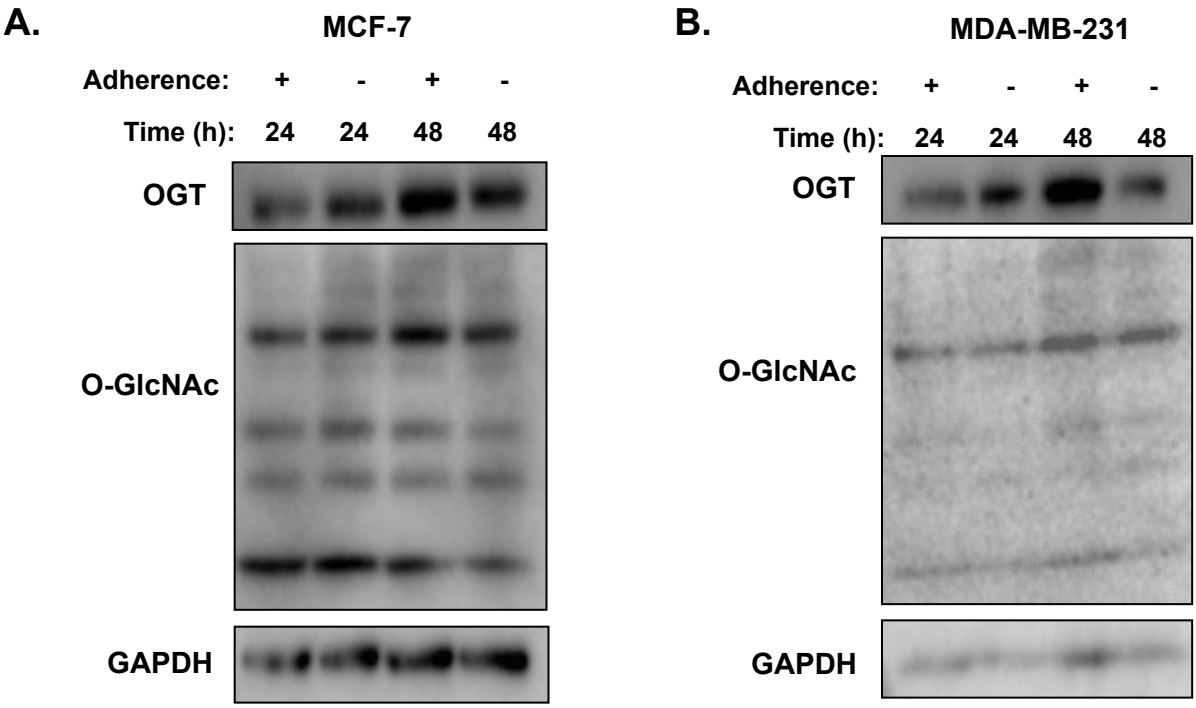
Supplementary Figure S10. Regulation of KLF8 by OGT in breast cancer cells. A. Cell lysates from MDA-MB-231 cells expressing control, OGT-1 or OGT-2 shRNA were collected for immunoblot analysis with the indicated antibodies. **B.** Cell lysates from SUM159 cells expressing control, OGT-1 or OGT-2 shRNA were collected for immunoblot analysis with the indicated

antibodies. **C.** Cell lysates from MCF7 cells expressing control, OGT-1 or OGT-2 shRNA were collected for immunoblot analysis with the indicated antibodies. **D.** Measurement of relative mRNA expression of indicated genes from control or stable OGT shRNA expressing MDA-MB-231 cells using qRT-PCR. All expression is normalized to cyclophilin A internal control and to MDA-MB-231 shRNA control samples. Student's t-test reported as mean ± SEM. * = p-value < 0.05.

Supplementary Figure S11. Breast cancer patients with high KLF8 have poor overall survival.

A. Kaplan-Meier overall survival curve for Luminal A breast cancer patients according to KLF8 expression levels. **B.** Kaplan-Meier overall survival curve for HER2-positive breast cancer patients according to KLF8 expression levels. **C.** Kaplan-Meier overall survival curve for Mesenchymal breast cancer patients according to KLF8 expression levels. **D.** Kaplan-Meier overall survival curve for Basal-like 2 breast cancer patients according to KLF8 expression levels.

Table S1. List of upregulated and downregulated genes.


A.
MCF-7
Adherence: + - + -
Time (h): 24 24 48 48
OGT
O-GlcNAc
GAPDH
B.
MDA-MB-231
Adherence: + - + -
Time (h): 24 24 48 48
OGT
O-GlcNAc
GAPDH

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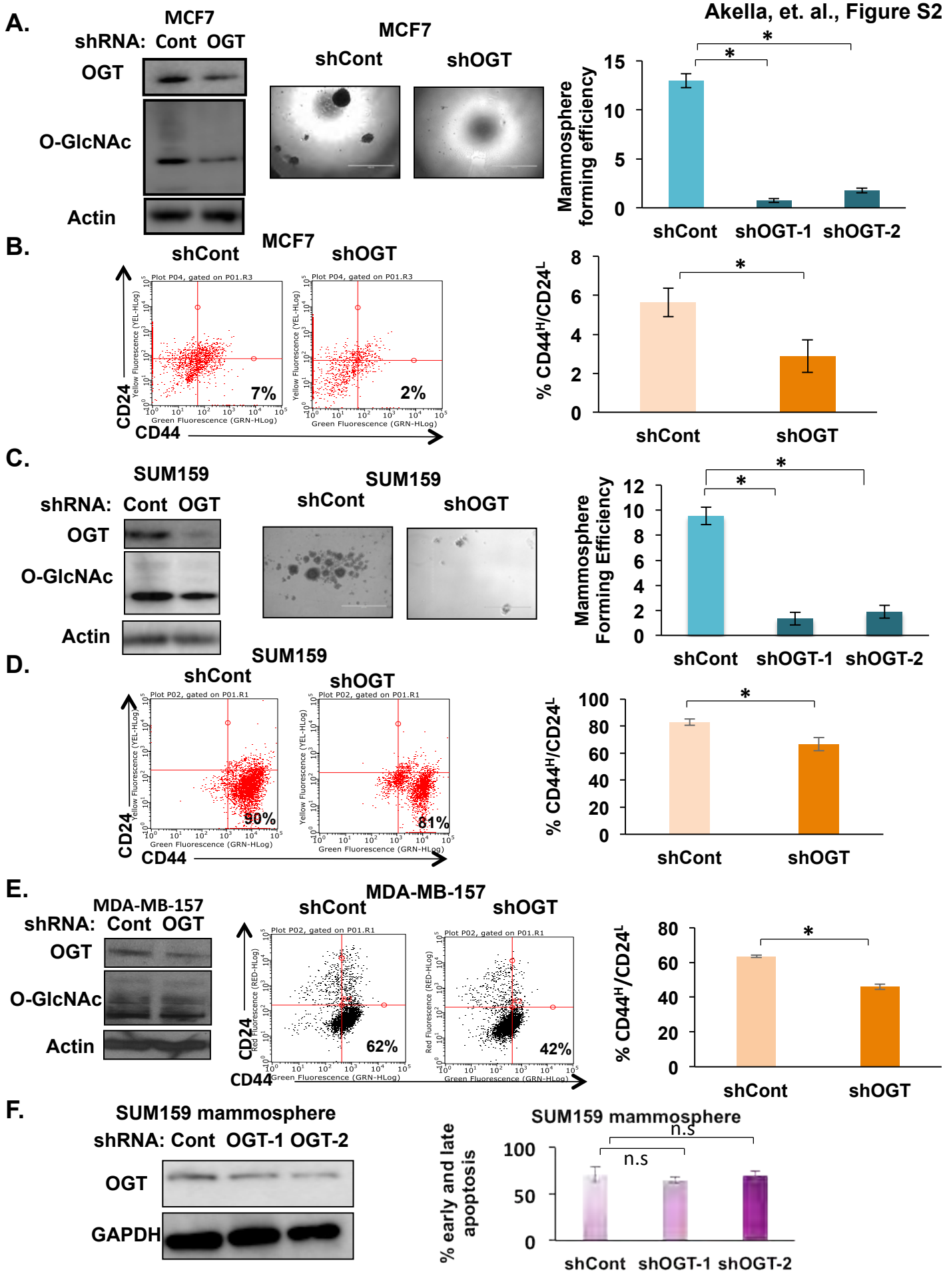
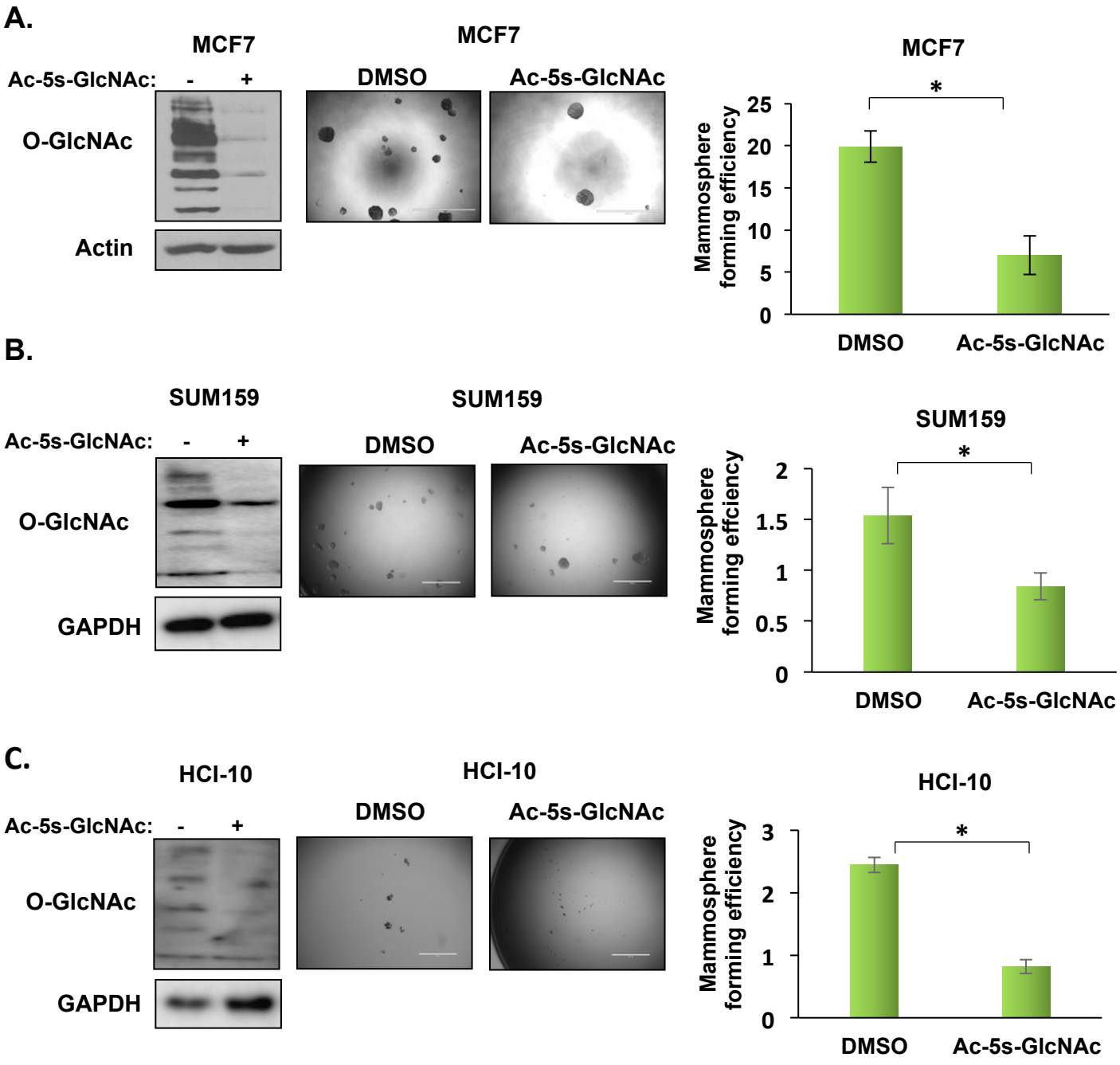
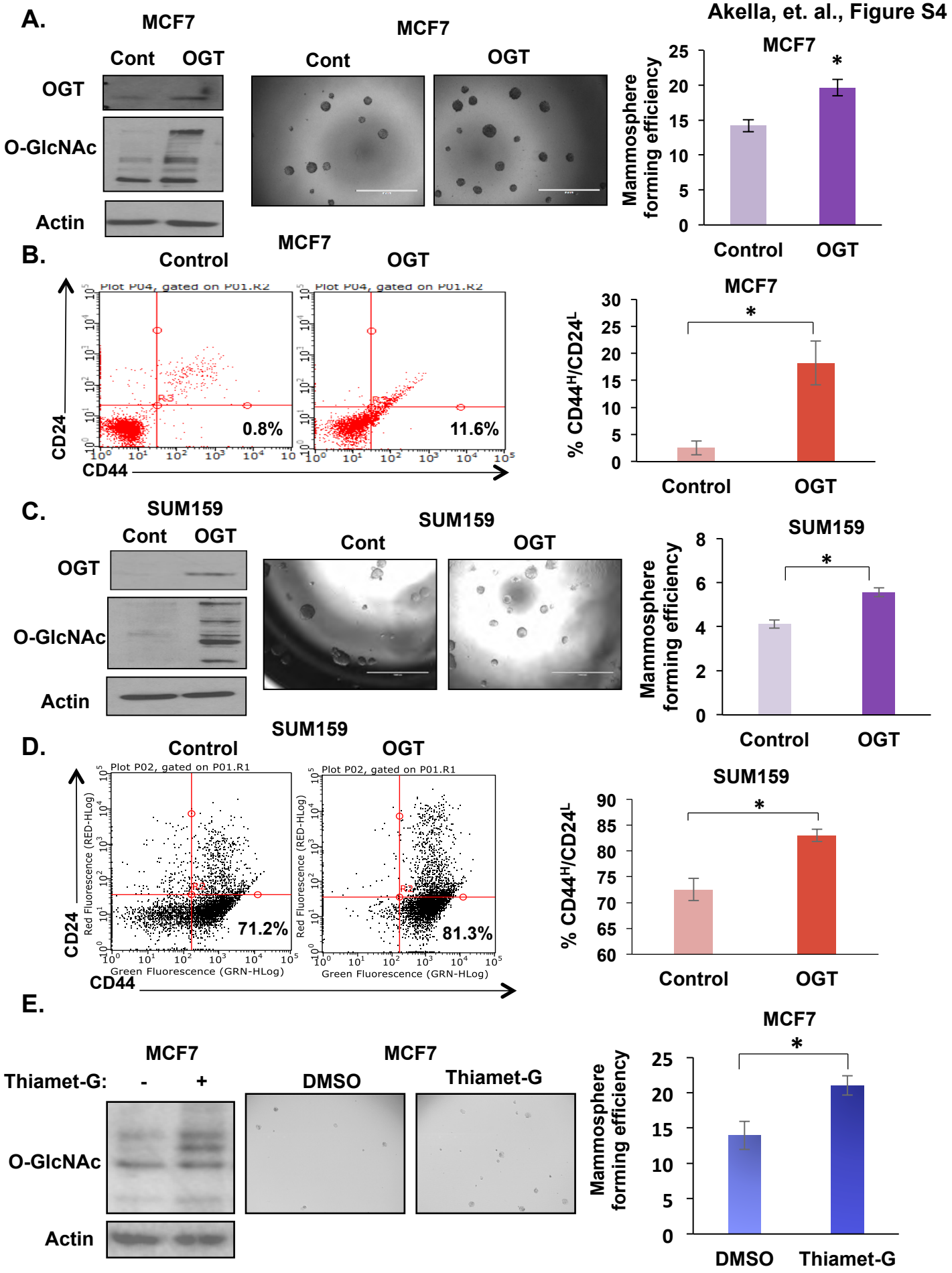
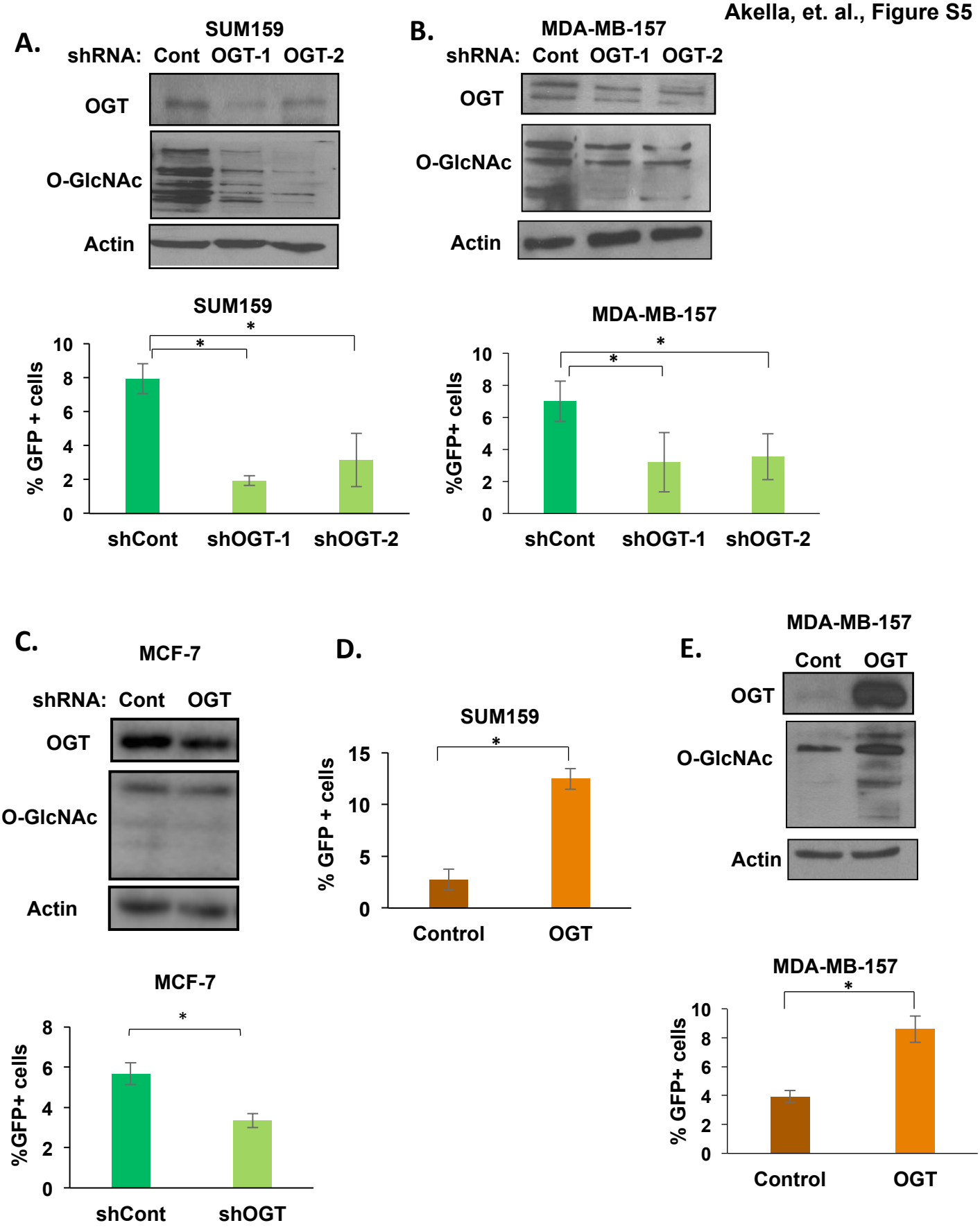


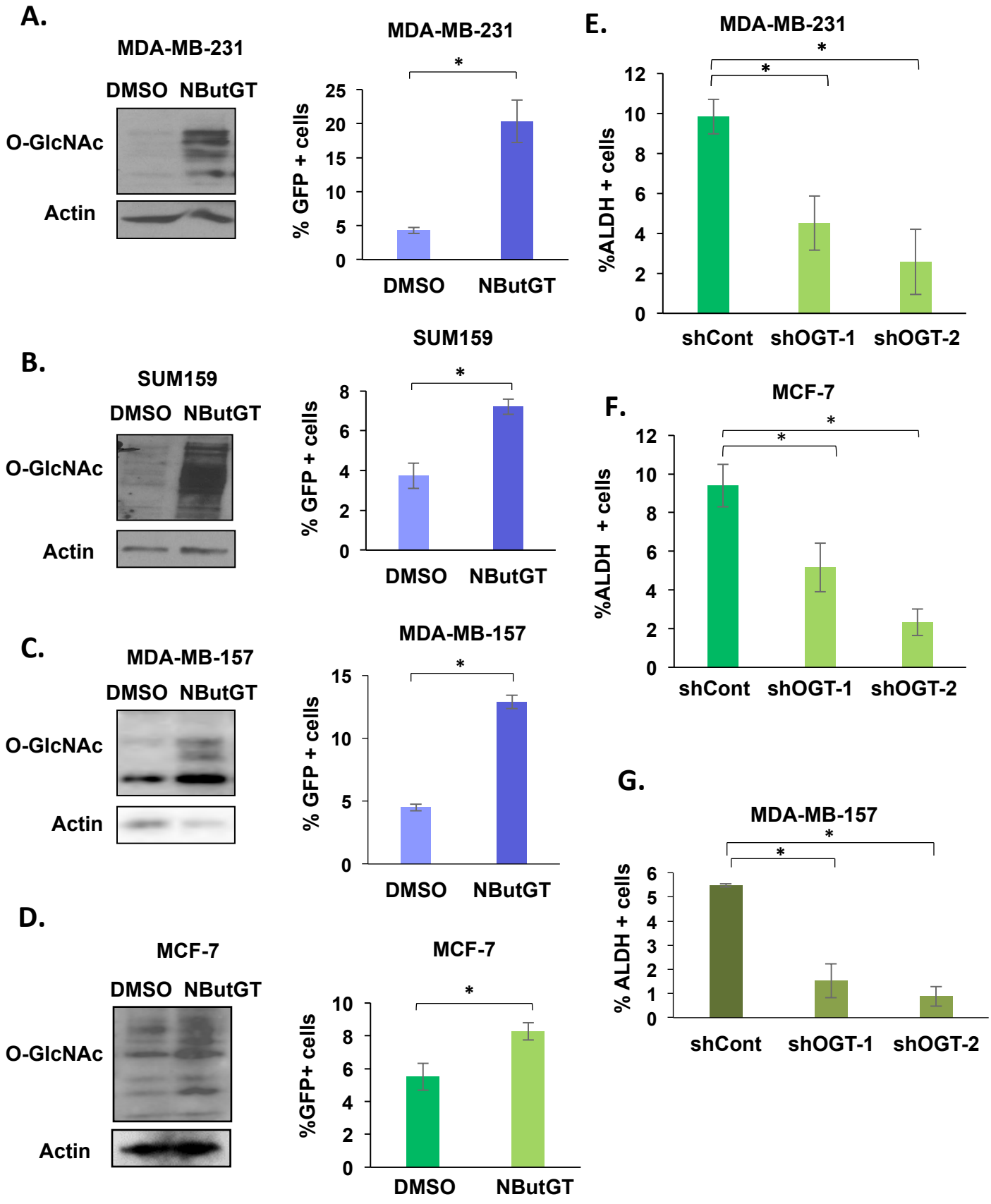

A.
MCF7
Ac-5s-GlcNAc: - +
O-GlcNAc
Actin
MCF7
DMSO
Ac-5s-GlcNAc
MCF7
Mammosphere forming efficiency
25
20
15
10
5
0
*
DMSO
Ac-5s-GlcNAc
B.
SUM159
Ac-5s-GlcNAc: - +
O-GlcNAc
GAPDH
SUM159
DMSO
Ac-5s-GlcNAc
SUM159
Mammosphere forming effciency
2
1.5
1
0.5
0
*
DMSO
Ac-5s-GlcNAc
C.
HCI-10
Ac-5s-GlcNAc: - +
O-GlcNAc
GAPDH
HCI-10
DMSO
Ac-5s-GlcNAc
HCI-10
Mammosphere forming efficiency
3
2
1
0
*
DMSO
Ac-5s-GlcNAc

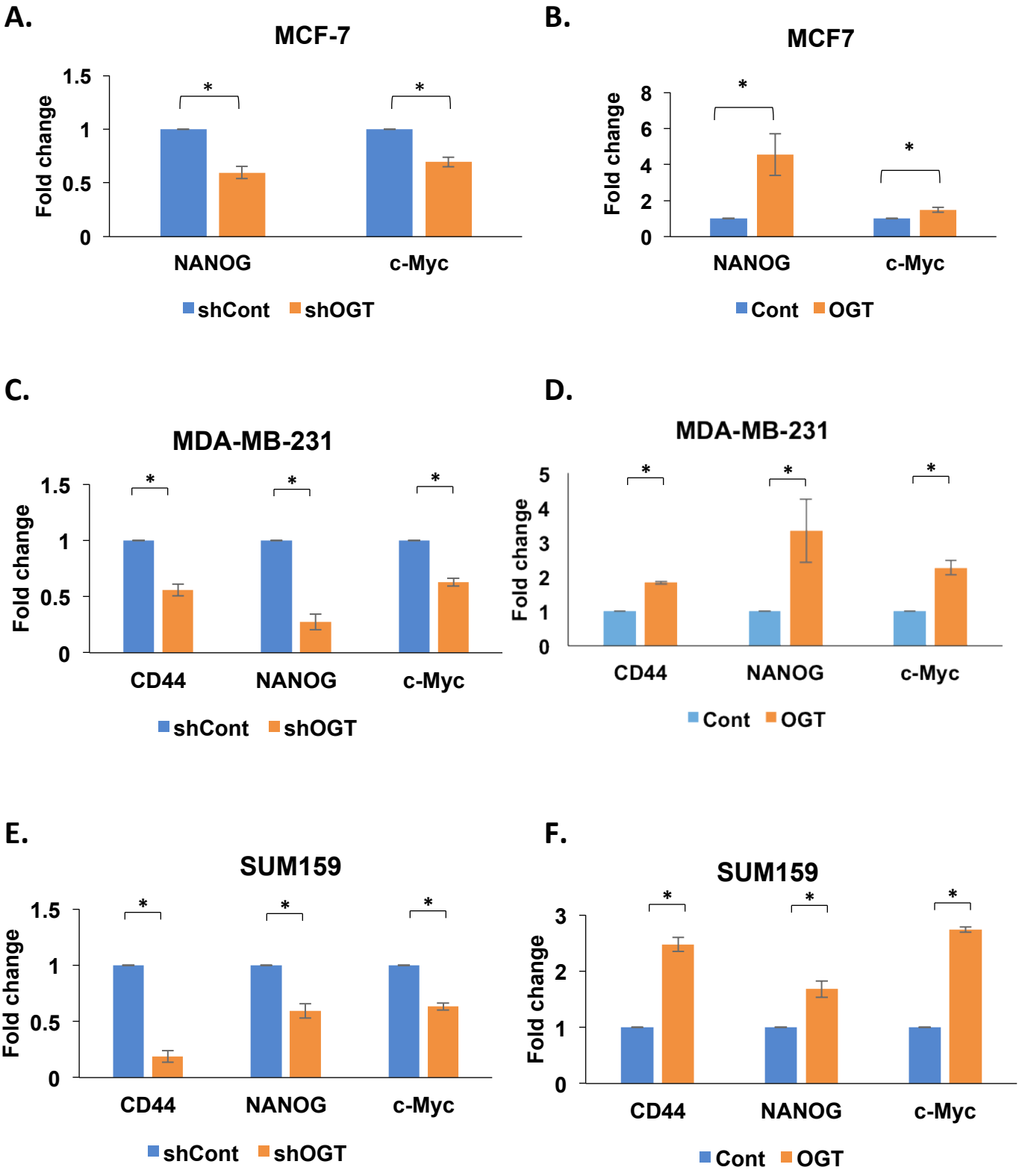
Akella, et. al., Figure S4

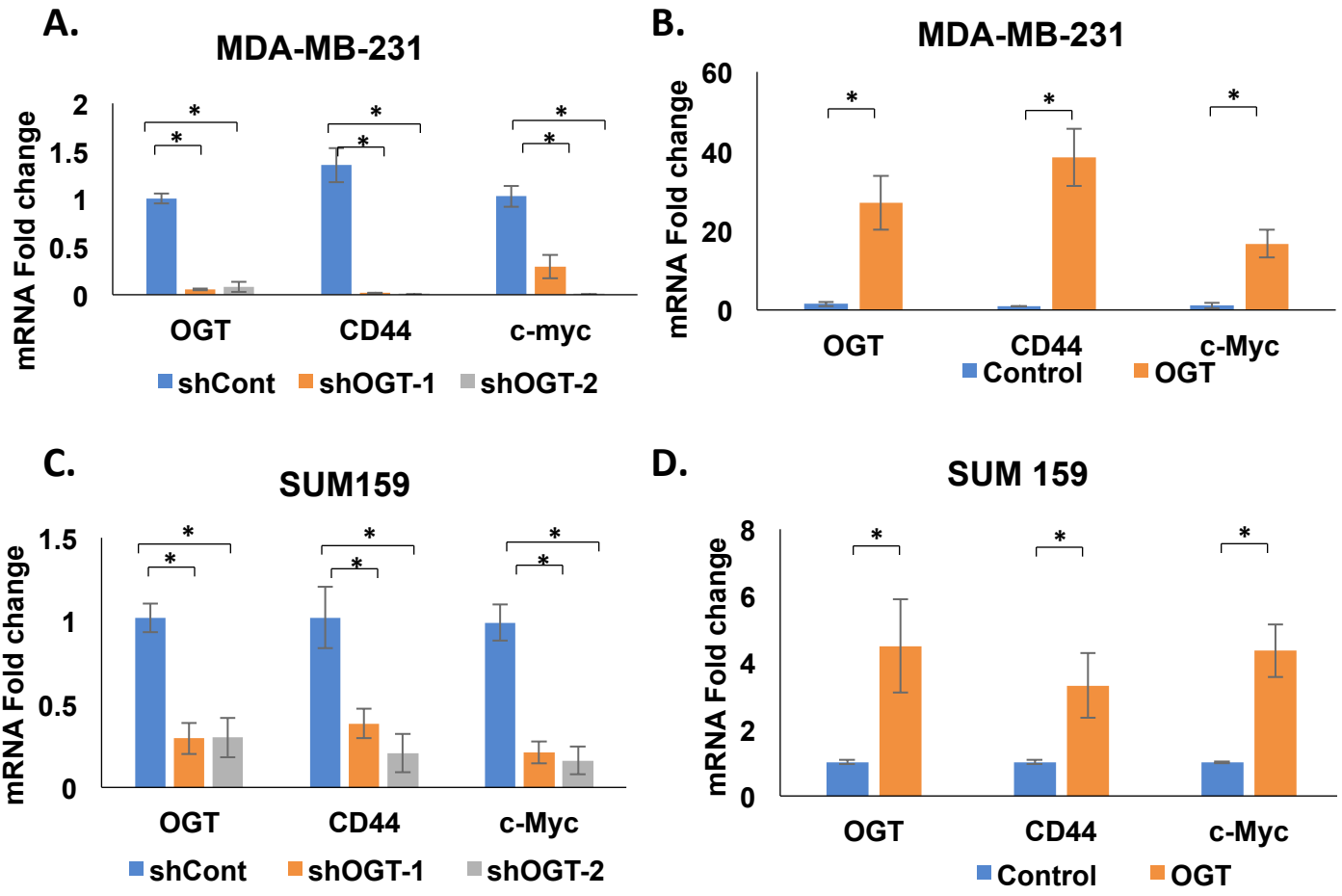


Akella, et. al., Figure S5

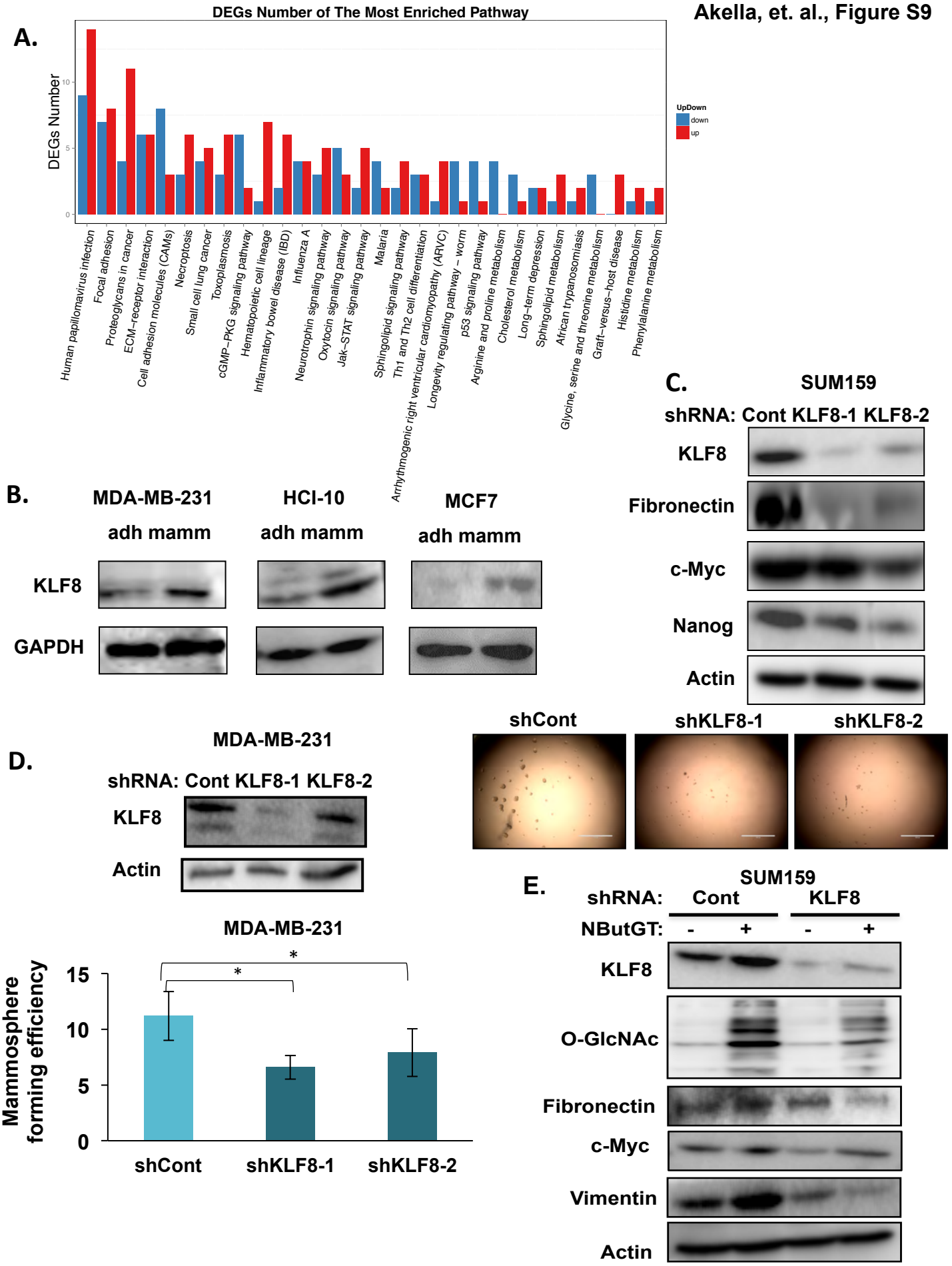



A.
MDA-MB-231
DMSO NButGT
O-GlcNAc
Actin
MDA-MB-231
% GFP + cells
25
20
15
10
5
0
*
DMSO
NButGT
B.
SUM159
DMSO NButGT
O-GlcNAc
Actin
SUM159
% GFP + cells
8
6
4
2
0
*
DMSO
NButGT
C.
MDA-MB-157
DMSO NButGT
O-GlcNAc
Actin
MDA-MB-157
% GFP + cells
15
10
5
0
*
DMSO
NButGT
D.
MCF-7
DMSO NButGT
O-GlcNAc
Actin
MCF-7
%GFP+ cells
10
8
6
4
2
0
*
DMSO
NButGT
E.
MDA-MB-231
%ALDH + cells
12
10
8
6
4
2
0
*
*
shCont
shOGT-1
shOGT-2
F.
MCF-7
%ALDH + cells
12
10
8
6
4
2
0
*
*
shCont
shOGT-1
shOGT-2
G.
MDA-MB-157
% ALDH + cells
6
5
4
3
2
1
0
*
*
shCont
shOGT-1
shOGT-2

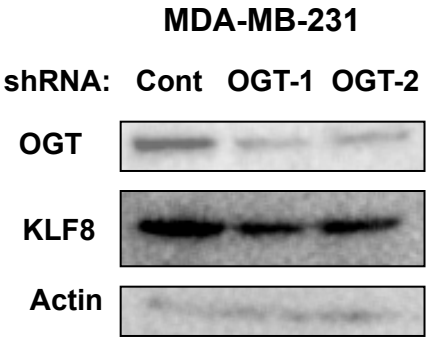

A.
MCF-7
Fold change
1.5
1
0.5
0
*
*
NANOG
c-Myc
shCont
shOGT
B.
MCF7
Fold change
8
6
4
2
0
*
*
NANOG
c-Myc
Cont
OGT
C.
MDA-MB-231
Fold change
1.5
1
0.5
0
*
*
*
CD44
NANOG
c-Myc
shCont
shOGT
D.
MDA-MB-231
Fold change
5
4
3
2
1
0
*
*
*
CD44
NANOG
c-Myc
Cont
OGT
E.
SUM159
Fold change
1.5
1
0.5
0
*
*
*
CD44
NANOG
c-Myc
shCont
shOGT
F.
SUM159
Fold change
3
2
1
0
*
*
*
CD44
NANOG
c-Myc
Cont
OGT


A.
MDA-MB-231
mRNA Fold change
2
1.5
1
0.5
0
*
*
*
*
*
*
OGT
CD44
c-myc
shCont
shOGT-1
shOGT-2
B.
MDA-MB-231
mRNA Fold change
60
40
20
0
*
*
*
OGT
CD44
c-Myc
Control
OGT
C.
SUM159
mRNA Fold change
1.5
1
0.5
0
*
*
*
*
*
*
OGT
CD44
c-Myc
shCont
shOGT-1
shOGT-2
D.
SUM 159
mRNA Fold change
8
6
4
2
0
*
*
*
OGT
CD44
c-Myc
Control
OGT

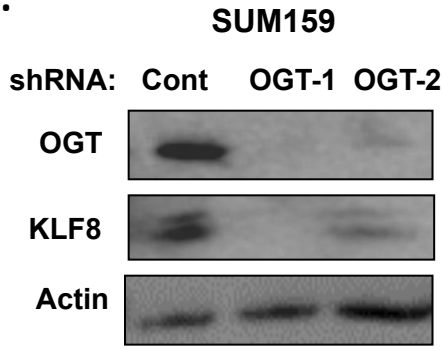
Akella, et. al., Figure S9



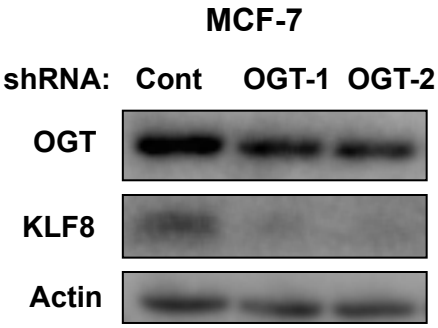
A.


MDA-MB-231
shRNA: Cont OGT-1 OGT-2
OGT
KLF8
Actin

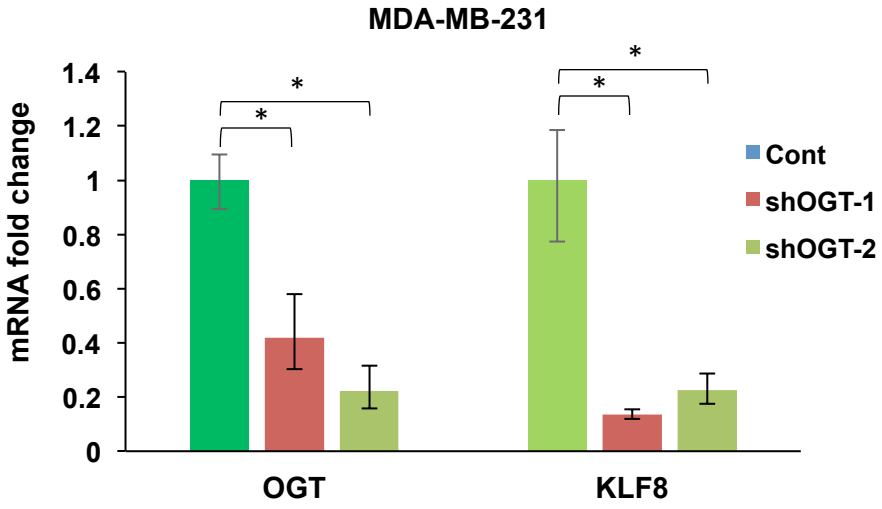
B.


SUM159
shRNA: Cont OGT-1 OGT-2
OGT
KLF8
Actin

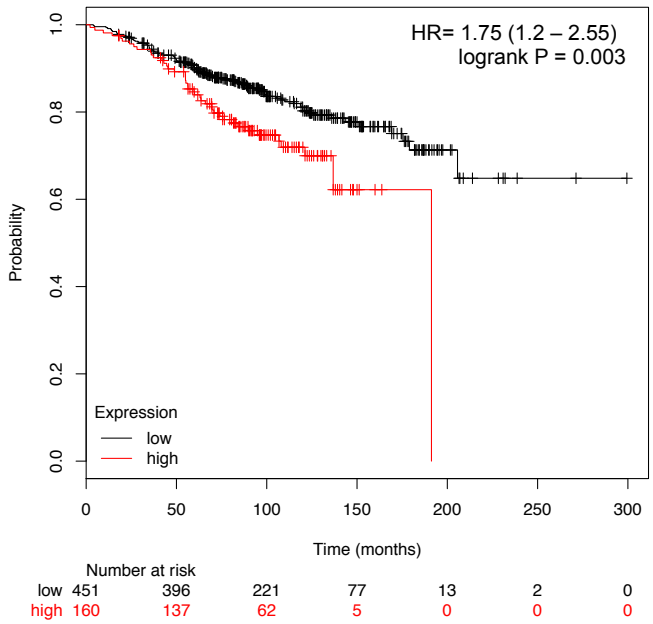
C.


MCF-7
shRNA: Cont OGT-1 OGT-2
OGT
KLF8
Actin

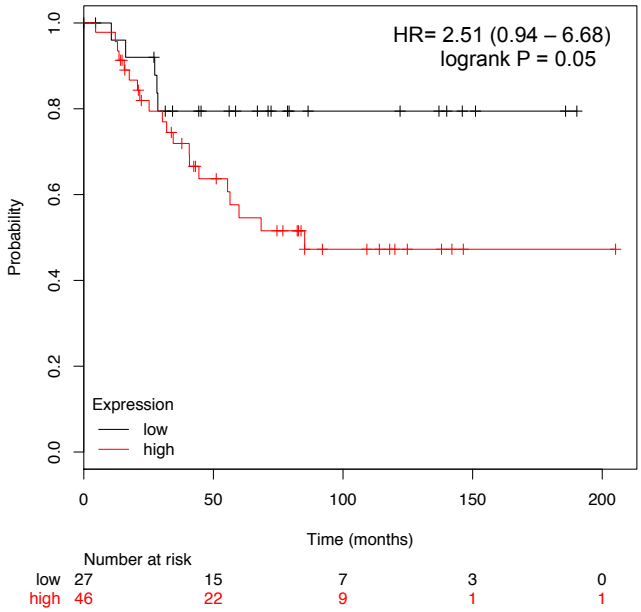
D.


MDA-MB-231
mRNA fold change
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
*
*
*
Cont
shOGT-1
shOGT-2
OGT
KLF8

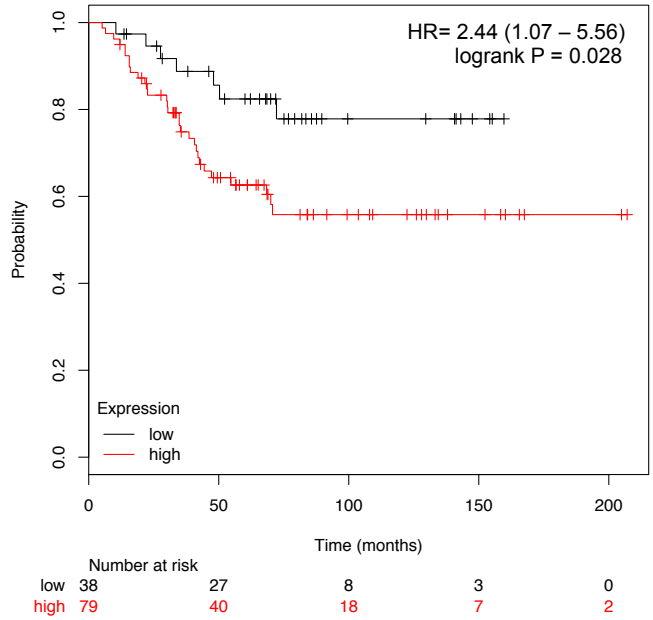
A. Luminal A breast cancer



C. Mesenchymal breast cancer



B. HER2-positive breast cancer



D. Basal-like 2 breast cancer